NetNotes

Edited by Thomas E. Phillips
University of Missouri

phillipst@missouri.edu

Selected postings from the Microscopy Listserver from September 1, 2010 to October 31, 2010. Complete listings and subscription information can be obtained at http://www.microscopy.com. Postings may have been edited to conserve space or for clarity.

Specimen Preparation:
glutaraldehyde

We currently receive our glutaraldehyde in 8% - 10 ml vials. We then buffer them down and aliquot to a 3.5 % solution for use in the EM lab. In hopes of maximizing our resources/time we are looking into purchasing glutaraldehyde already aliquoted and diluted. I have seen that there are vendors that supply 2.5 % glutaraldehyde in buffers. What is the opinion of the lower concentration and does anyone purchase these products? ***Sue Trant susan.trant@viha.ca Wed Oct 13***

The issue of glutaraldehyde aliquots at different concentrations, with or without buffers, is stability and efficiency of the fixative. With time, even when stored at low temperatures, glutaraldehyde tends to polymerize. These polymers decrease the fixative solution effectiveness. The higher the concentration, the more polymerization you will have. It also depends on pH, temperature, and age of reagent. Concentrated glutaraldehyde at room temperature polymerizes very fast when there are bases, acids, or oxygen present. If the solution is in low concentration, this is slowed down. These solutions contain mostly the monomeric (active) form of the fixative and are stable at pH 3–8 (as much as I can remember). If there is water in the solution, glutaraldehyde tends to polymerize. On the other hand, keeping working concentration of glutaraldehyde in buffers suffers changes in the solution osmolarity. With time, the glutaraldehyde solutions in buffers tend to increase their osmolarity, so you won't have equal fixing conditions with aliquots from the same lot in different time intervals. In general, if the color of the gluteraldehyde is getting yellowish, don't use it. In addition, if the pH is below 3, it's not good. What I usually do is buy small volume aliquots of unbuffered 25% glutaraldehyde (usually in ampoules), store them frozen and always prepare fresh fixative on the day of the experiment. Josif Mircheski jmircheski@us.es Fri Oct 15

In regards to the stability of 8% glutaraldehyde, Sigma Aldrich product data sheet states: "Purified samples of 8% glutaraldehyde stored at –20 C showed virtually no change in their UV absorbance characteristics even after 8 months" with this reference—Gillett, R., and Gull, K., Glutaraldehyde—Its Purity and Stability. Histochemie, 30, 162–167 (1972). See also Don Ranly's informative article on the stability of glutaraldehyde: http://www.aapd.org/upload/articles/Ranly-06-02.pdf. The amount of work required to dilute 8% glutaraldehyde is worth the effort. If you don't want to measure it out, design your protocol so that you use the entire ampoule at once. Tom Phillips phillipst@missouri.edu Fri Oct 15

A note regarding glutaraldehyde degradation: (a) In a carefully monitored study, a 25% aqueous solution of glutaraldehyde was purified to a single peak with a UV absorbance maximum of 280 nm (3). The subsequent detection of a second peak at 235 nm indicated the formation of alternate forms, such as polymers, in investigations of the influence of pH, temperature, and buffering on polymerization rate. No polymerization occurred when a solution of glutaraldehyde was stored for 5 months at –14°C. There was a slight increase with storage at 4°C, and then a rapid increase in this peak beginning with storage around 20°C continuing to 60°C. The polymerization rate of glutaraldehyde was increased when the pH was slightly acidic or basic; the rate polymerization was decreased somewhat by the addition of buffers. If a 50% degree of polymerization can be tolerated, samples may be stored at pH 6.5 for up to 7 months. (3) Rasmussen, K.-E. and Albrcchtscn, J., Glutaraldehyde. The influence of pH, temperature, and buffering on the polymerization rate. Histochemistry, 38, 19, 1974. (b) My experience in the tissue preservation for medical purposes at 4C at <1% glutaraldehyde buffered to 7.5–8 pH (sodium bicarbonate) will begin degrading in a couple of weeks and continue until not useful at 3 months. Tony Havics ph2@sprynet.com Fri Oct 15

Sorry if I did not read carefully, but I did not notice anyone pointing out yet that in the sealed ampoules, unbuffered glutaraldehyde is kept under inert gas. Therefore, it should last longer. Otherwise, my understanding is that lower concentrations of such unbuffered stock last longer than more concentrated ones, because polymerization rate is slower. There is a good discussion, with literature references, on the subject of glutaraldehyde fixation in Gareth Griffiths' Fine Structure Immunocytochemistry book. Vlad Speransky speransv@mail.nih.gov Fri Oct 15

In my experience, sealed ampoules of glut under nitrogen will last—as long as the ampoule itself does not leak. At least, I have not seen any difference that I can detect in the results. Fred Monson fmonson@wcupa.edu Fri Oct 15

As someone who worked in an EM service lab for many years, I am well aware of the problem of having people "dilute glutaraldehyde with buffer". If you dilute 8% glutaraldehyde with an equal amount of 0.2M buffer you get 4% glutaraldehyde in 0.1M buffer, which is fine. But many times clients diluted 25% or 50% glutaraldehyde with 0.2M buffer only, resulting in a strongly hypertonic solution which causes the tissue to appear very dense, obscuring ultrastructural detail and causing poor sectioning. Glutaraldehyde should be diluted to twice the desired end concentration with water, and then mixed with an equal volume of 0.2M buffer. I also remember reading somewhere that concentrated glutaraldehyde is more stable than dilute. Ralph Common

While there are several to many explanations for initiations of polymerizations of various compositions, the requirements for HCHO are unique, because the native state (at STP) for HCHO is a gas. While, again, I have not been able to sort thru ALL of the literature, my memory is jogged sufficiently to remind me that most studies of polymeric reactions involving HCHO do not address the self-polymerization of the substance. I have been in my present location for 10 years, and there are 3–4 packs of 10ml glass vials of 50% glutaraldehyde marked with the phrase, "sealed under nitrogen" or something to that effect (I am operating without perfect memory or immediate access to them. In the last 20 years, I have picked one and then another of these packages from colleagues who are moving or closing their labs. In all of that time, I have only experienced one

https://doi.org/10.1017/S1551929510001148 Published online by Cambridge University Press

loss of a vial to polymerization (there was no odor of 'glut' when I cracked it to deactivate the substance). To summarize what I now remember, I offer the following. Even in 50% aqueous solutions of glutaraldehyde or HCHO, the key to stability appears to be oxygen. Further, the polymerization can be photo-catalyzed. Thus, in addition to a nitrogen environment, my vials are made of brown glass. So, I suggest that by removing oxygen via nitrogen purging and keeping the substance (8–50%) in the dark, one may expect that the common initiator of reactions—free radical oxygen species—will not be created in quantities sufficient to initiate noticeable polymerization OR oxidation. The other characteristic of aldehyde polymerizations is low pH. HCHO + O → HCHOOH (formic acid). I have not used Formalin for fixation of any kind since the mid 1960's, since the day I was asked to tap a 55 gallon container and received a trickle of liquid and lots of white flakes. When I prepare HCHO from paraformaldehyde, I prepare 200 mls @ 20% (by weight), and I store it for no more than 6 months in 50–100 ml screw cap jars with PTFE-lined caps—sealed for id with a wrap of 'Parafilm'. Fred Monson FMonson@wcupa.edu Fri Oct 15

I have never quite understood the relevance of the concentration of the buffer components in glutaraldehyde or formaldehyde fixatives. No doubt there is an effect of the buffer, this has been amply illustrated. But if one considers the contribution of the aldehydes to the molarity of the fixative solution, it seems something else than tonicity is at play. Monomeric glutaraldehyde has a molecular weight of 100. A 4% solution corresponds to 0.4M. The situation is even more strange for formaldehyde with a molecular weight of 30. A 4% solution being 1.33M. Clearly at the very onset of fixation the situation is that the fixative is highly hypertonic. Perhaps it is rather the buffer capacity than the osmolarity that is important? Jan Leunissen leunissen@aurion.nl Wed Oct 20

My understanding is that they can cross the membrane and therefore don't count in the osmolarity. Tom Phillips phillipst@missouri.edu Wed Oct 20

Specimen Preparation:

stability of uranyl acetate and lead stains

Does anyone have any protocols for making uranyl acetate and, especially, lead grid stains that will reliably store for several weeks? Sometimes we go for quite a spell between needing to stain grids, and end up needlessly wasting batches of stain solution. I'd like to make up a batch of each and be able to store aliquots, if possible. (We're currently using 5% Uranyl Acetate (aq) and Reynolds' Lead Citrate, but are open to others.) ***Jaclynn Lett lettj@ent.wustl.edu Wed Sep 8***

I make up 10 ml at a time and store each one in a 10ml syringe, fitted with a 0.22 micron filter and cap. I cover the uranyl acetate syringe and filter with aluminum foil. I store them at room temperature and they last for weeks to months. When I'm using them I discard the first couple of drops out of the end, figuring it was on the wrong side of the filter. This has worked great for me for decades! Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Wed Sep 8

Uranyl acetate will last for several months if it is made in water and kept in the refrigerator. I have been using 2% for over 30 years but I do use a block stain of 1% UA before dehydration of the specimens. There is a slight sediment in the bottom of the bottle but I only take from the top and discard that in our mixed waste when I make a new batch. When I am ready to stain, I put a drop of 2% UA on Parafilm inside a Petri dish and add a drop of 100% ethanol, a grid and close the dish which has been covered to keep out the light. Ten minutes and it is ready to be water washed a few times then left in a drop of water in dish two before putting the grid into lead stain (no drying). Lead Stain X-from the 25th M&M meeting in Chicago in 1967. Ref. is on page 148–149 by Aly Fahmy. 50 ml water boiled and cooled into a 50 ml polypropylene test tube 1 dry NaOH pellet into the water, cap the tube and dissolve pellet completely. Add 0.2 to 0.25 grams lead citrate (tip of spatula) Invert tube until all is dissolved and clear. Let sit 15 min. in the dark for crystals to settle (if any). This works as well as Reynolds' lead citrate for me. Fill syringes (1 to 10 ml), attach an 18 gauge needle, expel air and stick into a large rubber stopper. Discard the last few drops left in the tube. Label with date. Put into the back of a refrigerator. These can last a year but as a rule I discard them after 6 months. Take out one at a time to use at room temperature. Stick the cold syringe into a smaller stopper in a dark cabinet or put a tall metal can over it and allow time to warm-up. Expel the first few drops in case of crystals or use a small syringe filter. Note: some 0.2 micron filters do not work the filtered stain will not stain! The syringe 'in use' is usually fine for a week or so if kept dark. I put out a few drops at a time on fresh Parafilm in a third dish and stain for 1 to 2 minutes. Wash well and dry. Excessive lead staining time will cause the stain to lighten, not get better. Patricia Stranen Connelly connellyps@nhlbi.nih.gov Wed Sep 8

Sato's lead is remarkably stable—I am still using the stock I made in November, 2008. This link has recipes: http://www.2spi.com/catalog/chem/sato_recipe.html. R. Howard Berg rhberg@danforth-center.org Wed Sep 8

Microtomy:

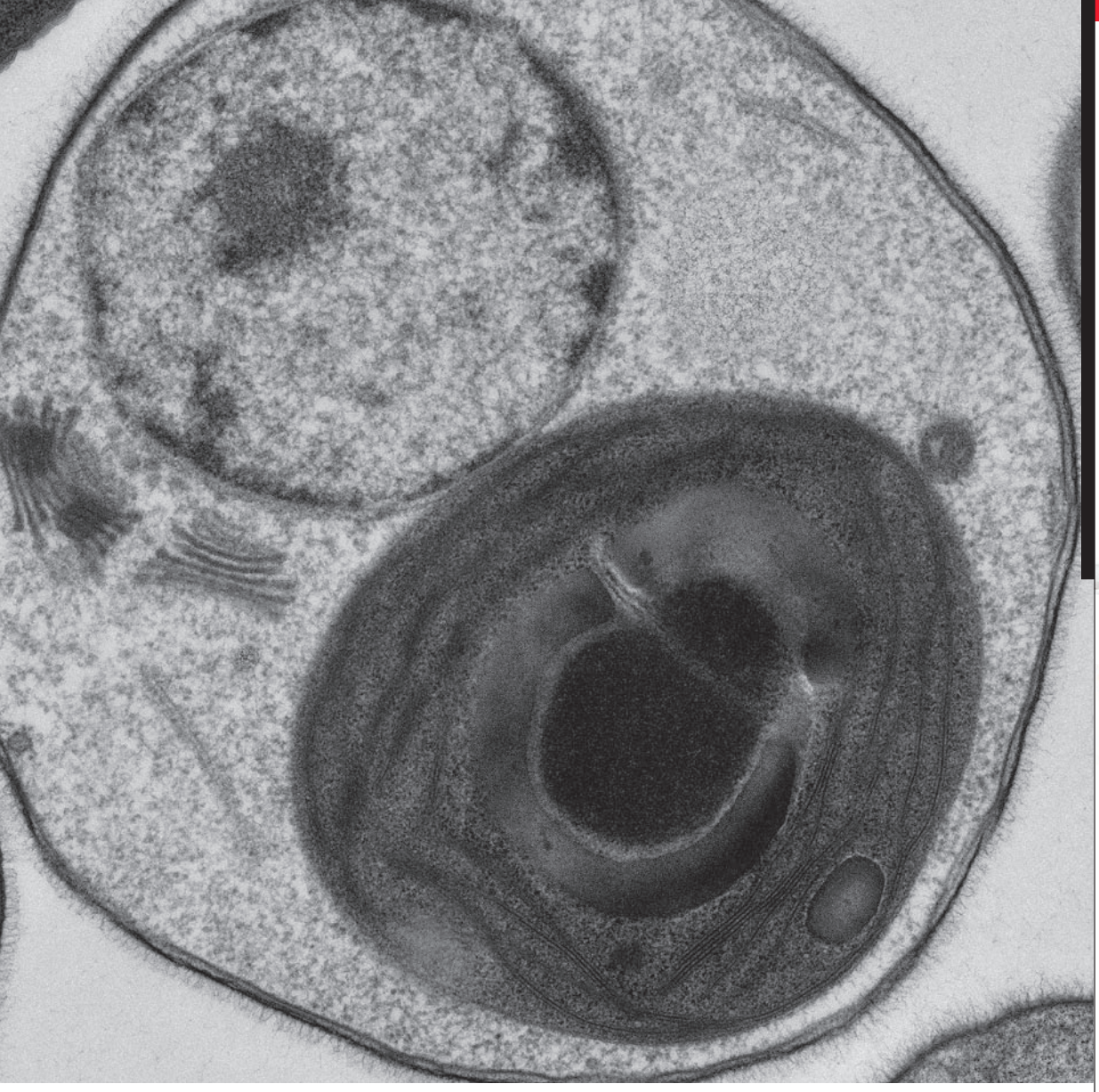
coated grids

Does anyone know where to purchase 50 mesh, nickel grids with Formvar coating? I know, you are thinking, this guy is too lazy to make his own grids, but we have been unable to get our films to strip from the slides for a long time. ***John J. Bozzola bozzola@siu.edu Wed Sep 1***

Two problems you may be having are, first, humidity—the lower, the better for films coming off—and, second, if you use nose grease on the slides, the person's nose grease may not have the optimal composition. My nose grease, for example, is iffy in that ordinary films will come off, but holey films stay stubbornly stuck to the slide. In the latter case, I dissolved a small amount of Apiazon L in petroleum ether and coated the slides with that, then the holey films came right off. I'm sorry I do not remember the amount of Apiazon I used. Bill Tivol wtivol@verizon.net Wed Sep 1

Rather than casting the films onto glass slides, try using freshly cleaved mica. I have seen many recipes (Formvar voodoo) for coating/polishing/treating glass slides, but have never had good success casting films on them. If you score the edge of the mica, the water penetrates easily and the films float right off. If you insist on using glass slides, I would opt for a detergent based coating rather than any kind of grease (nose or otherwise). The detergent should give less contamination since it dissolves in the water used for floating off the film. I've used standard lab detergent (no lemon scent added!) for coating ion mill windows and bell jars with good success. Dilute it a bit, coat the glass, and then polish most of the detergent off. The deposits usually come right off the glass with maybe a little bit of rubbing. After getting some silicone contaminated carbon films from a major EM supply house, I decided that I need to make my own. They aren't as pretty, but at least they're clean. Henk Colijn colijn.1@osu.edu Wed Sep 1

Bill mentioned the nose grease thing on the list server a number of years ago. Tried it. May work for him, it was a total flop for me. We had trouble off and on for years. Super cleaned the slides, put grease on them, etc. One thing I noticed is how recently the slides were made seemed to have an effect. Invariably, we have trouble when the box of slides is old. The second they start to go milky, have whitish spots, etc, we cannot get the slides off. But slides from a new box, recently



Antarctic Algae. HPF freeze-substitutes in 2% Oso4 in acetone. Sample provided courtesy of Dr. Kirk Czymmek and Shannon Modla, Delaware Biotechnology Institute Bio-Imaging Center

Your Image Starts Here!

Innovative Sample Preparation for a Wide Range of Applications

Leica Microsystems offers the most comprehensive product portfolio for precise preparation of high-quality biological and industrial materials samples for TEM, SEM, LM, Confocal, and AFM. Our instruments meet the highest expectations for precision and ergonomy in the field of nanotechnology.

Sample Preparation for Every Need

Sectioning, processing, staining, planing, target polishing, ion milling, contrasting, high pressure freezing, cryo processing and transfer, coating and drying are all expertly addressed by one or more of our innovative instruments.

www.leica-microsystems.com

Living up to Life

Leica

MICROSYSTEMS

https://doi.org/10.1017/S1551929510001148 Published online by Cambridge University Press

purchased, consistently will release the film. The other problem we've found is using slides that are super clean—acetone, ethanol, etc. But as Markus said, a slide fresh out of the box, wiped with a Kimwipe works best (actually, I use a lab coat sleeve, but then I've been accused of being some form of reprobate anyway). The second thing is how long the Formvar has been on the slide. Actually tried this silly little experiment just because I got curious as to whether I could make up a box of slides at one time, and use them over the next several months. Dipped slides and tried floating the Formvar off after 10, 15, 30, 45, 60, 90, 120 mins, 4, 8, 16, 24, and 48 hours. Did 10 slides each. Anywhere from 10 to 30 minutes was great, after that slides started to give a little trouble. About 75% of slides released the Formvar after 2 hours. By 16 hours, almost no slides released the Formvar. So making the film on slides in advance, and casting them into the water days or months later is just not going to work, at least in our hands. Also, what are you making them in? We use ethylene dichloride. Have seen other solvents recommended, never tried them. Make it up 100 ml at a time, pour it back into the Coplin jar after each use—now everyone can scream about contamination, bad technique, etc—but it works. Be practical, help limit the amount of pollution you put into the environment, and stay out of the clutches of your Health and Safety Office. We dip about 12 slides, and immediately pour the Formvar back into a bottle, seal with Parafilm, and wait to use it later. I have used it for up to 2 years. Having said that, I also always float off the first film, put a single grid on it, and then check that grid in the microscope immediately to make sure there are no problems with the Formvar solution before I make up 400–500 grids. We are still making up over 3,000 grids a year. Since the technician that was so good at making them became ill and passed away I have just made them myself. Too much trouble getting the other technicians to get it right. I make up 1200 at a time, and make up a batch every time we get down to 200. If your films are weak, just make a higher concentration solution next time. And if the lab you go into insists one plastic is better than another, go with the flow. Not worth the argument, and like politics and religion, you will never change a primal belief. Paul R. Hazelton paul_hazelton@umanitoba.ca Thu Sep 2

There is a lot of anecdotal evidence on the best way to remove Formvar films from the casting substrate. I was shown the "nose grease" method when I was starting out in the field. As I mentioned, mica seems to be much more reliable than glass slides as a substrate. Also, adding grease to the substrate runs counter to my practice of absolute cleanliness on my samples. Regarding Formvar films . . . I generally use Formvar only for my holey (not holy) or lacey support films since Formvar films are generally too thick for my purposes. A chemistry colleague (note the anecdotal evidence) told me that dichloroethane degrades with time forming hydrochloric acid. This degradation is accelerated by light. The upshot is that one should store the Formvar/dichlorethane solution in a dark bottle in a dark storage cabinet and make up fresh solution periodically. Certainly my films were much stronger with the fresh solution than with my old stuff. I use the huffing technique (ala Arte Johnson) after casting the films to create holes, rather than to serve as a separation layer. Since the moisture in your breath is immiscible in the dichloroethane solution, you get droplet formation and, after the water evaporates, holes. No garlic, escargot or butter required! Henk Colijn colijn.1@osu.edu Thu Sep 2

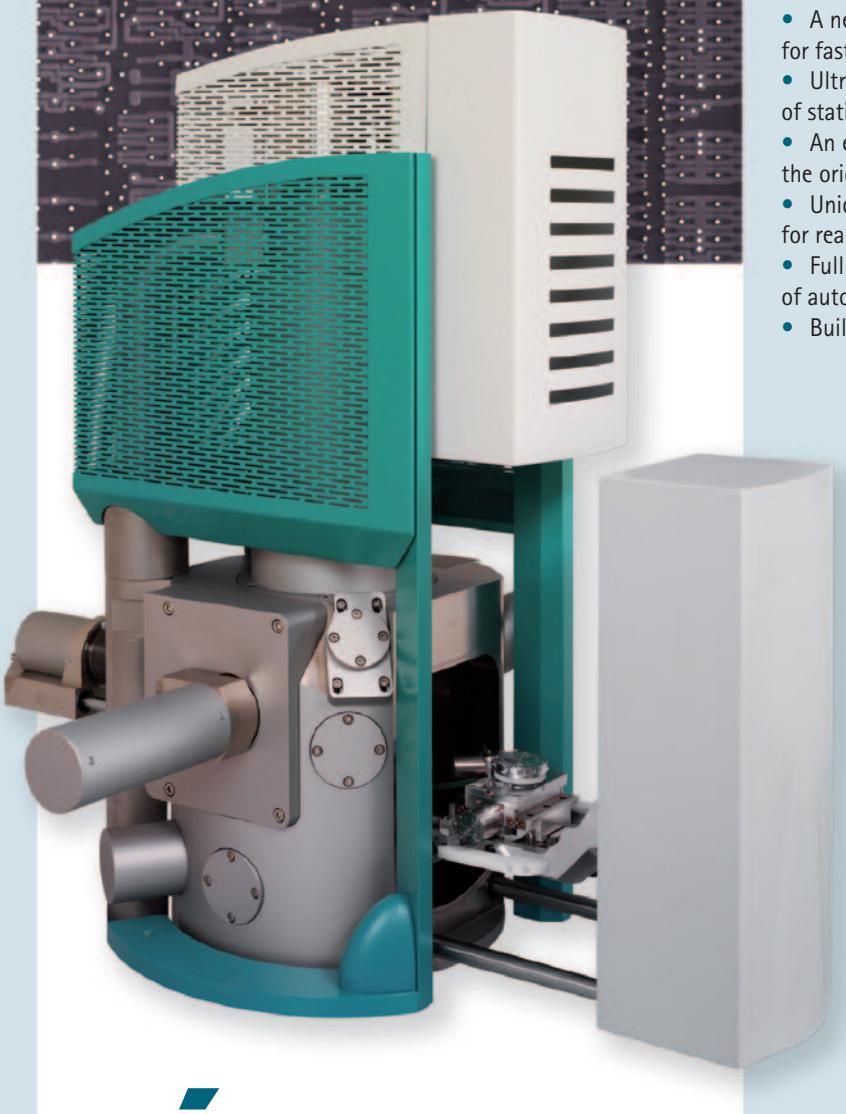
Everyone's got their tricks. Mine include the following: 1) I buy special-order 8% Formvar from Ladd then I dilute to about 0.8–1% to use (or whatever the math in #2, below, works out to). This seems to be our magic solution. I never make from powder any more. 2) I do this in a Dip-Miser beaker so that I only have to make about 11 ml (1 ml 8% Formvar plus about 10ml ethylene dichloride). 3) I have the Dip-Miser propped up in a beaker, nestled in desiccant in the bottom of a peanut butter jar, the metal top of which has a hinged trap door cut into it, beaker surrounded by gauze well moistened with ethylene dichloride. The top is held down by a dive weight when I'm not dipping—I actually think this is the crucial component ;-) 4) Our "magic slides" change every couple of years. The current box is three-year-old plain Corning slides that do not seem to get frosty as they age. I wipe not too well with Kimwipe. Have been known to use nose grease, Ivory hand soap, whatever feels right that day. 5) Because of the humidity here, I dip the slide, let the Formvar run off it a bit back into the beaker in the ethylene dichloride atmosphere (amount of time helps determine film thickness) with the trap door as shut around my hand as I can get it, then I quickly put it to dry on filter paper that is on top of desiccant in another jar. 6) Strip and float and add grids as usual. I pick up from the top with Parafilm which, once I tried it, makes me very, very happy. 7) In my hands, really old coated grids (>2 years, preferably about 6 years) are happily hydrophilic. Otherwise I fire up the Denton carbon evaporator with a bad leak which just happens to be the perfect leak rate for glow discharging the grids. This is my excuse for not repairing it. Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Thu Sep 2

An additional trick is to use hot water. When I was using Apiazon on the slides, I also prepared a solution of Alconox, 1 g/l and put it in the 70 deg oven. I used this in a staining dish when it came time to float the Formvar. Bill Tivol wtivol@verizon.net Thu Sep 2

I am a true recidivist, if that is the correct word or concept. Not sure. My intellectual EM parent must hate me. He taught me so well, where did I go wrong. Buy the ethylene dichloride from VWR. Could buy it anywhere. Throw in a 4A molecular sieve, again, from VWR. Not using the ethylene dichloride to dehydrate tissue for embedding, so there really are no concerns of microscopic particles of the molecular sieve getting into the tissue and wrecking the diamond knife. I had trouble with getting dry ethylene dichloride for years. Would get a new 500 ml bottle, use it once, and even with molecular sieves it would be bad the next time I opened it. Water in the solvent, leading to holy grids (not holey, but holy as in holy @##%@!!!). All sorts of holes. Then one summer day about 15 years ago I took a brand new bottle out of the explosion safe fridge to make fresh Formvar. In the summer the humidity tends to be high in Manitoba, not as high as Tina has to fight with, but high never the less, I came back 15 minutes later, after weighing everything out, getting the volumetric flask ready, etc., and picked up the bottle. It almost slipped out of my hand—coated with condensation. In a brief and rare flirtation with brilliance I realized that the problem of wet solvent was the result of proper storage—keeping the solvent cold. When it came out of the Fridge moisture in the air condensed in and out of the bottle, leading to wet ethylene dichloride, and an excellent medium for making holey (holy) grids. Not sure if there have been any such episodes of understanding since. Today I am using a 500 ml bottle that I bought 4 years ago. Keep it in the flammable storage cabinet at room temperature. Never have a problem. Probably will replace it next year since I do get worried about it being too old. As far as the breathing, it makes sense that if you immediately breath on the film when you take the slide out of the Formvar you should get some holes in the film. We wait until the film is dry—usually 10 minutes, but I suspect even 5 minutes would work. Have heard of using Parafilm to pick up the grids, never tried it. We pick up on a torn piece of paper towel. Let it get wet completely by wicking, and the film and grids stay down. If we don't let the towel get sufficiently wet, and the area of the film is not completely bordered by wet towel, then we find invariably that the film will not hold on the paper towel. Since we put 10–12 rows of 6 grids on a film, I really do not like having the film and grids slide off the paper towel—just because we tried to save 10–15 seconds.

Scanning Electron Microscopes

VEGA 3



Introducing New Generation of SEMs

After 10 years of continuous development, VEGA matured into its 3rd generation. The VEGA 3 series were designed with respect to a wide range of SEM applications and needs in today's research and industry and provides users an advantage of the latest technology.

Key features of the VEGA 3 Generation:

- A new high-performance electronics for faster image acquisition and signal processing
- Ultra-fast scanning system with compensation of static and dynamic image aberrations
- An extended range of scanning modes using the original Wide Field Optics™
- Unique Tescan In-Flight Beam Tracing™ for real-time beam current and spot size optimization
- Full software control and high level of automation without any mechanical adjustments
- Built-in scripting for user defined applications

TESCAN

PERFORMANCE IN NANOSPACE

Tescan USA
508 Thomson Park Drive
Cranberry Twp., PA 16066
Phone: 724-772-7433
Email: info@tescan-usa.com

www.tescan.com

In his post, Henk quite rightly points out something that I have also used as an explanation for why the new slides work better than old ones. It is my belief that the cheap surfactant or left over detergent on the slides from time of manufacture makes the removal process work. Over time this surfactant or detergent residue deteriorates, and is visualized as the milky white material on the slides. Of course, I have no hard data, so this hypothesis cannot be proven. Therefore, out of the awe and respect that should be shown to sauride (saurine? is that the right word or did I just make one up, oh well, dinosaur) electron microscopists, it must be given status of dogma—not? As far as degradation products, the MSDS sheet I have on file says it degrades to hydrogen chloride. Perhaps not HCl, but quite a nasty little compound on its own. Yep, keep it in brown glass. Paul R. Hazelton paul_hazelton@umanitoba.ca Fri Sep 3

Microtomy:
reusing grids

You know how we strive for perfection, right? Well, in my imperfect times, my hand coated Formvar grids are sometimes imperfect as well (popped coating in few or some grid spaces). This leaves me with quite a few grids that I "can't use". Does anyone clean and re-coat their grids? I read somewhere that chloroform is one way to remove Formvar. Does anyone else do this? Are there other solvents that I could use? Is this a bad idea? ***Andrea Calhoun acalhoun@bidmc.harvard.edu Tue Oct 5***

Yes, you could clean and re-use the grids. However, bent or warped grids should be avoided since a new Formvar film would not adhere very well. Or, you could use the uncoated grids to collect sections directly. John Bozzola bozzola@siu.edu Tue Oct 5

Ethylene dichloride (dichloroethane) works to clean grids. This is generally what Formvar is dissolved in when you buy it in solution. Randy Tindall tindallr@missouri.edu Wed Oct 6

Because we use between 2000–3000 Formvar coated grids I have followed this thread with some interest. About 15 years ago I considered whether it would be feasible to clean and reuse grids. Then I did the math. Grids cost $21.50/100, or about 21.5c/grid. This is all you save. Balance that against your time, the cost of reagents, and the risk that you may not get the grids clean. The ultimate result could be that you have to re-prepare, or even lose important specimens. It's really not worth it. If you want to talk sometime privately about preparation methods give me a call. We have a very low failure rate (below 1%) for Formvar coated grids. Paul Hazelton paul_hazelton@umanitoba.ca Wed Oct 6

I make my own carbon-coated grids, using collodion to make the initial film and dissolving it later in chloroform. If I botch a batch, I clean the grids of collodion in acetone. Just throw them in a beaker with acetone and ultrasound for a few seconds. Mary Fletcher maryflet@interchange.ubc.ca Wed Oct 6

Specimen Preparation:
soft blocks

Does anyone have a suggestion on what to do with blocks that are too soft to cut? These were embedded in LX-112 and for some reason they did not harden. Is there any way that this experiment can be salvaged? Thanks in advance. ***Georgianne Ciraolo Georgianne.Ciraolo@cchmc.org Mon Oct 25***

I worked with LX-112 a long time ago on peripheral nerve tissue. Sometimes there was enough attached fat on the sample that it impeded good polymerization. Depending on how soft your blocks are, I have sometimes had good luck putting some of the catalyst on a swab, smearing it on the surface (already trimmed and faced) and putting the block back in the oven overnight. It tends to firm up that surface enough for decent sections, as long as you don't go in too far from the surface. Douglas W. Cromey dcromey@email.arizona.edu Mon Oct 25

I've used LX-112 for years, love it, and have rarely had problems with it. So saying, I now have gummy bears in the oven! I suspect the catalyst is the culprit in this case. Often when it gets old, addition of it to the resin makes the resin darker orange. In this case the resin didn't darken at all, which made me suspicious. After two days in the oven at 60°C I was still getting fingerprints in the surface. I turned the oven up to about 75°C, which helped make them harder to the fingernail, so we'll see. In addition to upping the temperture, I have also known people to try UV and, in one desperate case, one friend froze the blocks and quickly sectioned them before they thawed. I can't remember if it was –20°C, –40°C, or colder, but too cold and they would be brittle. Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Mon Oct 25

On occasions we have had soft blocks. We have been able to rescue them by trimming away most of the gooey resin, soaking in propylene oxide for an hour or 2 (if the tissue is robust enough you can apparently sonicate for a short period in propylene oxide; Bauman and Mendell (1974)) but I have never done this. After soaking in propylene oxide re-infiltrate with increasing resin mixture in propylene oxide again followed by polymerization. This has worked for us in most cases. This method came from Principles and Techniques of Electron Microscopy, Biological Applications, by M.A Hayat (3rd Edition) page 133. Bauman and Mendell's paper is from Stain Technology 49:119 (1974). They were using Spurr's, we have used this method with some success with Agar 100 epoxy resin. Allan Mitchell allan.mitchell@stonebow.otago.ac.nz Mon Oct 25

Microtomy:
alternatives to water in trough

I am trying to section hygroscopic material, so I cannot use water. Any suggestions for liquid I can use for this? I have tried ethanol, however, the surface tension does not seem to be high enough, as the sections do not slide off the knife, or if they do, they are crumpled and slide beneath the liquid surface. ***Michael Behr mjbehr@dow.com Mon Sep 27***

Glycerol has been used in the past. I have tried it and it is a messy job. I believe RL Ornberg was the one who originated the approach with some work he did with Tom Reese. I don't have the original papers any more but my thesis lists "Ornberg, RL & Reese TS (1980) A freeze-substitution method for localizing divalent cations: examples from secretory systems. Fedn. Proc. 39(10):2802–2808" which I think is the correct reference. Tom Phillips PhillipsT@missouri.edu Mon Sep 27

As to my knowledge, in some circumstances also DMSO has been used as trough liquid for ultrathin sectioning: cf. Histofluorescent labeling of catecholaminergic structures in rotifers (Aschelminthes) Keshmirian J and Nogrady T Histochemistry and Cell Biology 89:189–192, ". . . concentration of DMSO for sectioning was 4%, and markedly improved the quality of cutting . . ." http://www.immunologie-labor.com/cellmarker_files/fach_ultracryo_1.pdf Kuhlmann WD & Viron A (1972) Cross-Linked Albumin as Supporting Matrix in Ultrathin Cryo Microtomy, J Ultrastructure Research 41:385–394 ". . . DMSO and sectioned on 50% DMSO in the trough at about –50°C. Negative stain . . ." http://www.diatome-knives.com/knives/cryo_knife.aspx Cryo Diamond Knife: ". . . The triangular holder, suitable for dry sectioning, as well as the trough, for sectioning using fluids (DMSO/water), are both made from a special copper-nickel alloy, . . .". Warning! DMSO readily penetrates skin and may carry other dissolved chemicals into the body. May cause eye, skin, and respiratory tract irritation. Combustible liquid

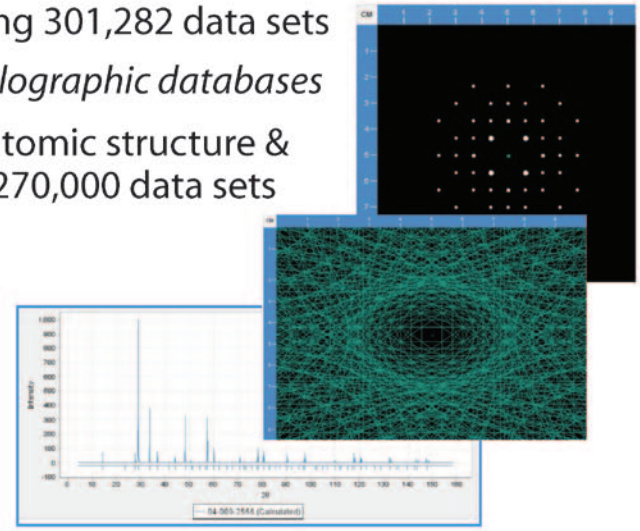
CONFIDENCE COMES WITH GREAT RESULTS

PDF-4+, an Electron Diffraction Database

The most comprehensive collection of inorganic powder patterns

Phase identification using physical, chemical and crystallographic data

- Comprehensive materials database featuring 301,282 data sets
  - *Standardized, edited data from four crystallographic databases*
- Electron Diffraction simulations based on atomic structure & electron diffraction scattering factors on >270,000 data sets
  - *Electron diffraction powder pattern*
  - *Interactive spot pattern display*
  - *Electron diffraction backscatter pattern*
- 147,393 data sets with atomic coordinates
- Atomic and molecular visualization
- Phase identification using elemental composition
  - *All formulas converted to both atomic and weight percentages*



ISO 9001:2008 QUALITY ASSURED COMPANY CERT.NR. 11409.1

ICDD® International Centre for Diffraction Data

International Centre for Diffraction Data
12 Campus Boulevard • Newtown Square, Pennsylvania 19073-3273 U.S.A.
Phone: 610.325.9814 • Toll-free: 866.378.9331 (U.S. & Canada) • Fax: 610.325.9823
www.icdd.com • www.dxcicdd.com

and vapor. Hygroscopic (absorbs moisture from the air. Target Organs: Central nervous system, eyes, skin; e.g. cp. http://fscimage.fishersci.com/msds/07770.htm. Dimethyl sulfoxide (DMSO) is a clear hygroscopic liquid; melting point 18 C; . . . It is miscible with water; readily soluble in almost all organic solvents. Wolfgang Muss w.muss@salk.at Mon Sep 27

I haven't done this, but years ago a colleague had embedded plant samples that were destined for EDAX and the component of interest was water-soluble, so cutting with a regular water-filled boat was out. She was able to cut the sections dry, then pick up the (crumpled) sections and place them on drops of ethylene glycol on slides. She then stretched the sections with toluene vapor (the old stick trick) and dried them down on a hotplate/slide warmer. My notes say that she actually dried them with heat in the presence of toluene vapor, but that would probably make your safety department cry or have a collective nervous breakdown, so maybe skip that part! Tamara Howard thoward@unm.edu Mon Sep 27

EM:

service contracts and insurance contracts

Our TEM was serviced once last year for preventative maintenance and a new camera meter. We paid almost $10,000 for our service contract. This is a large sum of money for an old scope that does not see much wear and tear from too many users. Does anybody have a more reasonable service contract for preventative maintenance and/or limited service calls for a reliable, but old film TEM? ***Barbara L. Plowman bplowman@pacific.edu Sat Oct 2***

This is a major issue facing many of us: contract or no contract. The advantages of a contract are obvious and the lack of one is simply a gamble. I do believe that older instruments, being of simpler construction and not using computers running Windows software, are less prone to breakdown. The older instruments could probably run indefinitely, except for the lack of replacement parts. By way of example, we have a 28 year old instrument that has been under service contract since the beginning and a 15 year old instrument that was never under service contract other than the first year warranty when it was purchased new. The 28 year old is running as well today as it did on day one, while the 15 year old has been non-functional for 2 years due to the lack of a transformer I am told. Even if we had the 15 yr old on a contract, the part would still not be available. So, in the latter case, a contract would have been of doubtful value. The money we saved by not having coverage would be enough to purchase a new instrument. Our plan now is to search for the part from a used instrument and bring the instrument back on line. In both instances, we made the right decision. My personal preference is to always have coverage if the money is available. If not, then very carefully control the use of the instrument and do the basic maintenance yourself. Call in the manufacturer's technical support staff when you cannot fix it or for annual preventive maintenance. $10K per year for a TEM seems reasonable, but you could half the cost by calling in service once a year or as needed. I hope others on this listserver will be able to provide some names of service providers in your area. If not, stick with the manufacturer, but on an as-needed basis. John J. Bozzola bozzola@siu.edu Sat Oct 2

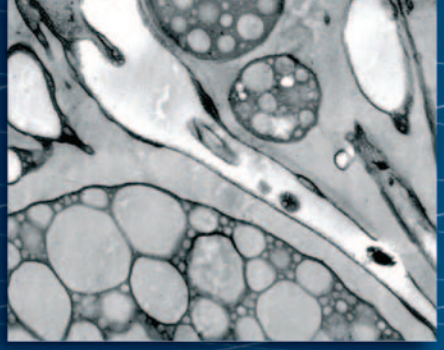
I strongly agree with those who argue in favor of having major instruments on a manufacturer's service contract. Note that there are independent service providers also who, by all accounts, do a very good job—I just don't have any personal experience with them. Without a contract, you are at the mercy of luck and the service providers' hourly rates for service and travel, plus expenses. Two or three days of this and you have already spent as much as a contract would cost. Except for one disastrous detour into the realm of insurance company maintenance coverage, we've had contracts on our scopes for forever. Same goes for ancillary equipment, which often seems to be neglected. In my experience, it is much harder to get administrators to agree to contracts for smaller pieces of equipment (coaters, microtomes, etc.), but the running of a facility is as much dependent upon them as upon the major instruments. Obviously, if you can't prepare a specimen to get it into a $500,000 microscope because a $50,000 piece of equipment is down, what good is the scope? Service costs for support equipment can be just as high as for a major piece. Case in point: we recently spent about $10,000 to repair a piece of equipment that had a service contract cost of about $5000. Costs included $350/hr for travel, which involved an engineer driving from quite a distance. Travel costs alone were ~$3000. The engineer was here for about two hours and did an excellent job incidentally, then had to order a part which I then later installed myself. On the other hand, you can always take the chance and hope that nothing goes wrong. Randy Tindall tindallr@missouri.edu Mon Oct 4

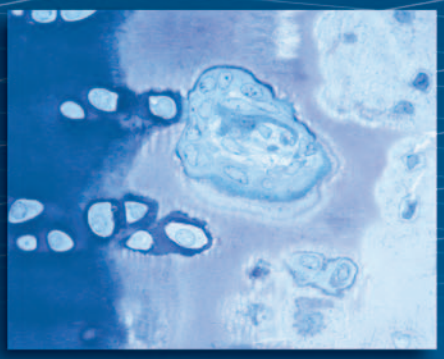
Another aspect that I see as an independent, and I'm sure the manufacturers see the same, is that a non-contract instrument often accumulates many small problems over time. They are ignored, or lived with, because administrators are loath to spend the money on a "minor" problem or even on "preventive maintenance". When the system eventually goes down, all they are willing to pay for is enough to get the system so it is not "down". Rather a moving target, don't you think? Anyway, in these instances the customer is often not satisfied (user, not administrator) and from a service engineer's standpoint it is very unsatisfying. Our goal is to have things work correctly, reliably and to specification. A service contract allows us to do what needs to be done without having to watch the clock. Sadly, if we do our job well, I often find that the microscopes end up having not much more than their 2 preventive maintenance calls per year after a few years because the major issues have been resolved and those, often quick, preventive visits still can turn up and fix problems that the user hasn't seen, yet. The bean counters look at that and say, "The contract is costing much more than those 2 calls per year. Cancel it and just schedule the preventive maintenance." Of course, the preventive maintenance calls aren't scheduled because "it's running fine so we don't need to spend the money." It's enough to make you pull your hair out. Fortunately, mine still grows back. Ken Converse kenconverse@qualityimages.biz Mon Oct 4

The conversation has been repeated over and over again and, although we run maintenance courses, there is in my mind nothing better than to have a professional electron microscope maintenance technician look after your instrument. The greater the number of instruments of the same make that you maintain the more you learn about them and the easier they become to fix. Trying to be a casual maintenance technician is tough. May I add a poignant comment from a retiring service technician in South Africa—"If your washing machine breaks down who do you call? If your television breaks down who do you call? If your computer breaks down who do you call? If your electron microscope breaks down who do you call, no you fix it yourself!!??" This has always amused me. Steve Chapman protrain@emcourses.com Mon Oct 4

I am also a big believer in service contracts and appreciate Steve's often insightful comments on the listserver but I will critique his logic here. A service contract isn't like calling someone to fix your washing machine or TV. First, you call after the breakdown—most people avoid pre-paying for service contracts on those devices. Second, a lot of broken TV's sadly need to be treated as disposable items these days since repair guys won't waste their time on smaller sets. It just isn't cost effective. My thesis lab had an old Seimens 101 and no money for a service contract. It was a difficult to keep running but we did

the highest quality...
the most precise sectioning...
incomparable durability





Free customer service

Sectioning tests with biological and material research specimens of all kinds. We send you the sections along with the surfaced sample, a report on the results obtained and a recommendation of a suitable knife. Complete discretion when working with proprietary samples.

Re-sharpening and reworking service

A re-sharpened Diatome diamond knife demonstrates the same high quality as a new knife. Even knives purchased in previous years can continue to be re-sharpened. The knives can be reworked into another type of knife for no extra charge, e.g. ultra to cryo or 45° to 35°.

Exchange service

Whenever you exchange a knife we offer you a new Diatome knife at an advantageous price.

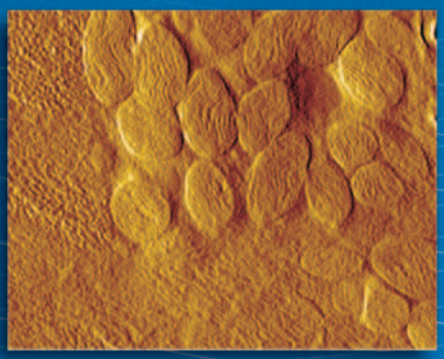
40 years of development,
manufacturing,
and customer service

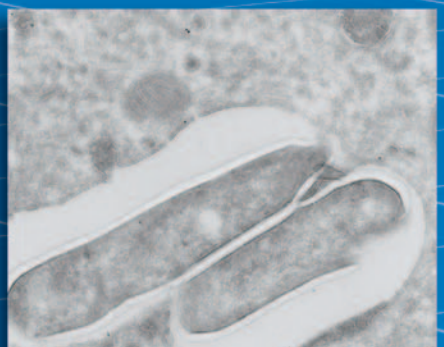
DiATOME

diamond knives

ultra 45° • cryo • histo • ultra 35° • STATIC LINE II
cryo-P • cryo immuno • ultra sonic • cryotrim 45 and 25
ultra AFM & cryo AFM • cryo 25°

Electron Microscopy Sciences
P.O. Box 550 • 1560 Industry Rd. • Hatfield, Pa 19440
Tel: (215) 412-8390 • Fax: (215) 412-8450
email: sgkcck@aol.com • www.emsdiasum.com





it—my brilliant thesis adviser even machined new parts for it one time. But I run an LM core now and would be up a creek without service contracts on my confocals. Tom Phillips phillipst@missouri.edu Mon Oct 4

It was not my quote but I did think it was apt. People do not fix their household items but expect to fix an instrument that is far more complex than all these household items put together. Would you agree? Steve Chapman protrain@emcourses.com Mon Oct 4

I am not in full agreement. Lots of scientists, by necessity, are quite mechanically adept. Several years ago, my paraffin tissue processor went out and the company wanted a 2K+ purchase order before they would come to fix it. Instead, my associate Mike Stanley, now of Chroma, and I took it apart and found a small motor that was broken. The company wanted $1200 for it. Instead, we looked at the motor and called the part manufacturer and got the same thing directly for less than $300. I remember once having my quite expensive Codonics color printer go out. We had no money for a repair so I told my young tech to take it apart with great care. She had never fixed a scientific instrument before this but spent two days disassembling it and photographed every single screw as she removed it so she would be able to figure out how to re-assemble. 2 days later she had repaired it and I don't ever remember her being more proud. Lots of scientists would think nothing of re-wiring their house, building a deck, or laying a tile floor such as I have done. Clearly fixing an EM is sometimes trickier but I would guess at least 20% of the time the problem is mechanical and could be fixed by a clever individual. But having said all that, I think almost everyone should have their EMs and confocals under service contract. I can think of a dozen times you have given brilliant advice to some listserver poster with an EM problem that enabled them to fix a problem—even with a service contract, one often needs to do some minor stuff. For example, your advice on HV discharge or maintaining rotary pumps earlier this year. I think your postings are some of the more knowledgeable ones on the listserver and usually select them for the NetNotes column in *Microscopy Today*. I fully agree with your conclusion on this issue but differ in the underlying logic. Thomas E. Phillips phillipst@missouri.edu Mon Oct 4

One factor not discussed so far is the impact of computer controlled instruments via a PC. This plus surface mounted ICs makes for a very difficult repair situation in comparison to simple embedded microprocessors. Coming from long Amray SEM experience, their earlier systems had socketed ICs and came with full schematics and components layout. These were very reliable tools and easy to fix. Later generation tools are "fixed" via board replacement. This is not a trivial issue and as such, a contract is important—if not very important. Some makers do not supply schematics, regardless of whether they would be of use. Gary Gaugler gary@gaugler.com Mon Oct 4

We could get by with just an annual PM and perhaps an emergency service call every couple of years if the service manuals for the instrument were available. I don't mind repairing our TEM; it's the best way to learn the inner workings. But it is impractical to attempt any serious repair by reverse engineering. My impression is that the service engineers usually follow step-by-step procedures outlined in their service manuals, which include electrical schematics, etc. for more extensive diagnostics. Fortunately, most of the companies seem willing to provide some phone support, but the manual pages are closely protected. If it would save $10K, I would learn to fix my washing machine, too, but I would still want the service manual. Not much of our research relies on uninterrupted access to a finely tuned washing machine, though. Phil Ahrenkiel phil.ahrenkiel@sdsmt.edu Mon Oct 4

Though my experience is not directly with our SEM, we presently have a similar issue with our EDS unit in our corporate climate. We dropped the service contract on our EDS unit a long time ago for substantial savings. I was proud that I could save the company money, reminding them that we could apply this money to replace the unit when the time came. Well, the time has come, and our EDS unit is in dire need of replacement. As you can probably expect, the company now says that there is no money for replacement. Where is all that money I saved them? We are stuck limping along with our old unit. I strongly recommend maintaining a service contract for those working in a corporate, and perhaps academic, climate. In the past I worked in a small independent lab where I was handy and the lab head was also the one paying for everything. In that case it made sense for us to have no or minimal service contract. Stu Smalinskas smalinskas@yahoo.com Mon Oct 4

Yes, we've had very good value out of our service contract on a confocal microscope, which includes all parts. On the other hand, we were quoted $40,000 for a service contract on an SEM, which seemed excessive to us (more than the confocal contract and did not include parts, but did include unlimited emergency visits—the contract for just two service visits a year was less). A service contract from the same company on light microscopes, while reasonable, does not include travel—fares and time, or overnight accommodation. Since we are not a hub for any of these companies, we're always slugged for travel. May not seem much each time, but we've had trouble with these microscopes, and if you have a few emergency visits a year it adds up. So there are good and not so great service contracts. Rosemary White rosemary.white@csiro.au Mon Oct 4

I wondered if I would dare go against all this wonderful community unity for service contracts from major companies. Well I still don't know if I'd dare, so let me be careful and make some math caclulations. For our TEM at least, the service contract would cost about 10% of the price of the microscope yearly. That means, with the money spared you could buy a new scope every 10 years or every 13 years, if you include the first 3 years under guarantee. This calculation was confirmed by a colleague of mine who runs a FEG under service contract. Now the question is: are 10/13 years so much for microscope? Are they so old after 10 years, that they need major repairs? Of course some pieces need replacement: ion getter pumps and the like, but perhaps with a little bit of training one can do it without the need for a professional. These pieces have a cost, but it was clear from previous posts that work hours and travel took a big part in the bill. I don't even want to think that after 20 years of service contract I could have 2 microscopes instead of a 20 years-old one even under service contract. Am I over-optimistic? Stephane Nizets nizets2@yahoo.com Tue Oct 5

Depending on the instrument design and the types of samples being analyzed, maintenance, even the routine (to be expected) stuff, can become quite time consuming which comes at a price even if you maintain the equipment yourself. Not to mention the infrequent system failures that crop up throughout the useful life of the instrument. I get paid to deliver results, that's the bottom line, the more time I spend learning how to inefficiently repair equipment the less efficient I am at meeting performance expectations. Now I can go hourly for routine stuff which would save us 20–30% off the contract rate, but any failures would eat into those savings. I would also risk losing priority status with the manufacture which would further cut into up-time and efficiency. To contract or not to contract . . . that is the question? And the answer is . . . it depends on your situation but in our case given our equipment, samples, staffing, and priorities, a service contract is the answer. I have a 20 year old FEG that works better than the day it was installed. If you do the math

SO MUCH MORE THAN MICROSCOPY.

HOOKE COLLEGE™ OF APPLIED SCIENCES

Hooke College of Applied Sciences is offering you more microanalytical training opportunities in 2011 with the addition of six new courses. Taught by industry leaders with decades of experience, our courses emphasize hands-on learning using state-of-the-art instruments.

Regardless of your industry or experience level, we can help you gain the skills and knowledge to successfully identify microscopic particles.

Interested in expanding your scope? Take a closer look at our 2011 courses at **www.hookecollege.com** or call **630-887-7100.**

UNLIMITED SCOPE in Training and Education

ANALYSIS · EDUCATION · INSTRUMENTS · a member of THE McCRONE GROUP

it was much cheaper to keep it under contract. John Robson john.robson@boehringer-ingelheim.com Tue Oct 5

These figures sound reasonable to me, Stephane. Based on an informal poll I made some time ago, it appears the life expectancy of a new EM is about 15 years though some would say 10–12 years. I was shocked by this figure, since I have worked with some perfectly fine instruments nearly twice that age. The older generation of instruments was simpler, with mechanical adjustments, relatively simple circuitry and no computer control. With the introduction of computerized controls for convenience of operation, and a booming economy, there was a paradigm shift to what we have presently. With the economic challenges we face, I believe OEMs should consider reintroducing a generation of simpler, reliable, less expensive instruments. Most biologists, especially at smaller colleges or research institutions, really do not need the burden of keeping high end instruments running, especially in a teaching situation. Most EM work is not being done at the highest resolutions, certainly in the biological area, so the high end instruments are really over-kill. There is an obvious need for high end instruments in some situations, but not everyone needs or can afford to drive a BMW when a Camry would fulfill most of their requirements. Does anyone remember the Philips 201? Imagine that instrument with a reasonably priced, digital camera! It can be done, for sure, and my hope is that some enterprising company would make this step. John J. Bozzola bozzola@siu.edu Tue Oct 5

I'd say you were over-optimistic. What administrator is going to allow you to accumulate that "10% savings every year" for 10 years? And then spend it on a new instrument, instead of writing a grant for the money? Unless you own your own company, meaning you can make such a decision, any money you save by not buying a service contract is very likely to be spent elsewhere by whoever does own the company or runs the university/institute, etc. Plus, I'd also suggest shopping more. I've been recently pricing TEMs, and service contracts from most companies are 8–12% of the purchase price—but not all. One EM manufacturer in particular is much lower. Less than 1/3 of the other companies by actual $ quote for the same coverage. And from experience, I'd rate their service as the best. A major reason for this is business models: most EM companies service departments are for-profit companies or divisions, with profit targets. The service department of the lower cost company is not, hence the lower price. Caveat: since I'm Tech Ed for *Microscopy Today*, I don't want to mention companies. But it's easy enough to check this—get quotes from sales reps. Or listen to them waffle when you try to get a quote, which is even more fun. And . . . this applies in the US, possibly also Canada. Europe, I don't know. Phil Oshel oshel1pe@cmich.edu Tue Oct 5

I'm right with John on this one, especially if the 201 has the optional side-entry gonio stage. Computer-control is nice, but I really wish the manufacturers would design instruments that can be run from a web browser. An Ethernet plug is pretty much compatible with anything. Julian Smith smithj@winthrop.edu Tue Oct 5

I agree with Julian about having something easy to control from any machine, however based on my extensive IT experience, I can assure you that once you plug an Ethernet cable into something you leave it wide open for all sorts of problems. Imagine if you had your remotely operated TEM, without a local console, compromised by a hacker who decided to "Exercise" the beam current and start blowing filaments. Personally, I've always been a fan of computer control of things with an isolated machine, but allowing for manual takeover if needed. I don't think an instrument should be permanently tied to a specific computer. I've seen way too much instability in both individual computers and computer technology as a whole. Justin A. Kraft kraftpiano@gmail.com Tue Oct 5

An equipment insurance company will be visiting us in the near future in an attempt to talk us into getting a policy through them rather than the manufacturer's service contract, which we have had for 40 years with great success and working relationship. I am sure the insurance might be ok for some equipment, but not SEMs and TEMs. I am looking for any rationale I can use to help explain why the insurance route is not the way to go, and to stick with our maintenance service contracts. Anecdotes or any experiences with insurance on EMs will be greatly appreciated. Mark Grimson mark.grimson@ttu.edu Tue Oct 26

With an insurance plan and I assume a pay-as-you-go service, you may find that the cost of replacements parts may be much higher than when under service contract. Typically when under contract the service engineer's time charges and the manufacturer's parts are at a discounted rate. Will insurance give you preventive visits as well as quick response for emergency visits? If your machine is very old a third party service contract may work, but I would not chance it on newer equipment. Roseann Csencsits rcsencsits@lbl.gov Tue Oct 26

No insurance company has yet invented a magical way of up-keeping complex particle beam instruments without the proper preventive maintenance and qualified service. Neither have they a way of outsourcing service to a vendor from central China or rural India @ US$2/Hr, to create money for covering their own overhead expenses and making profit without passing these extra costs to you, the end user. One way or another, as direct expense and/or as cost of extended down time when something serious happens to one of your instruments, service insurance arrangements will ultimately get you into paying for overhead expenses and profits of the insurance company—that is in addition to the costs of parts, labor, travel, and overhead expenses of the service organization (most likely the same OEM as you are using now). I clearly understand why service insurance arrangements are good for the equipment insurance company, but why and how could it possibly be good for the end user is beyond my (limited) comprehension. As an institutional lab your safest bet is to stay with OEM service contract, unless you and your boss are prepared to deal with political implications of alternative approaches. If you are really desperate for some cost reduction, then probably the second safest bet is to get service contract from one of the reputable third-party SEM/TEM service providers, there are few of them on this list. Valery Ray vray@partbeamsystech.com Tue Oct 26

An insurance policy is nowhere near the same as a factory maintenance contract, IMO. No way. I have never had an insurance policy other than for natural hazards (but these can be covered) and burglary, vandalism and business interruption and liability. When talking about an SEM or TEM, it requires a very deep breath. As Dirty Harry said, "Do you feel lucky?" OK, a HT tank fails and needs replacement. A nominal $14K item but zero with a maintenance contract. The odds of this happening? Greater than zero. It has happened to me. Later model SEM HT tanks do more than just HT . . . they do HT, filament current, extractor current, SE HT and other high voltage sources. So there are multiple points of failure here. Any one will take your tool off-line. Without a contract, this will cost you time and money. Oh, no contract? You are at the bottom of the priority list. So good luck. Some makers offer different levels of coverage and support at different costs. Evaluate these based on your usage load and up-time history. Also, carefully consider the state of the art of the tool. The more modern it is, the more you will need a maker's contract. Sad but true. More technologically advanced tools lead to more opportunities for failures but also offers favorable user interfaces and novel features. Gary Gaugler gary@gaugler.com Wed Oct 27

Be conscious that the insurance company will require you to make a yearly maintenance service by the manufacturer and given

that you have no service contract with them the maintenance will cost a lot. Stephane Nizets nizets2@yahoo.com Wed Oct 27

Whenever this question arises, I feel duty-bound to chime in. Stay with your manufacturer or a qualified independent service provider! Our two-year experience with insurance companies was disastrous. Virtually none of the promises they made were kept, such as "You will get the same level of service as you do now", or "You won't have to worry about paperwork". It was somewhat cheaper, but at what a cost. It once took 9 months to arrange for a preventive maintenance visit on one of our scopes. We had to justify parts replacement and service calls above a certain dollar level. We had to wait in line behind the customers who had OEM service contracts until the companies could fit us in. Etc., etc., etc. Once back on service contracts, all of these problems disappeared, and we again received the same excellent level of service we had received before—no arguing over needed parts, no extended waits for service, no extra levels of bureaucracy to go through. Stay with your OEMs or trusted independent. If yours are half as good as ours (JEOL, Hitachi and FEI), reward them with a little loyalty and it will pay off. Randy Tindall tindallr@missouri.edu Wed Oct 27

We currently have an insurance company (IC) as a middle man between us and our microscope company (MC). This is the way it works . . . We pay the IC to supply us with a service agreement at a price lower than what the MC charges. The agreement matches what the MC would supply (this is important) including preventative maintenance visits. How it is supposed to work is that when we have a service need, we call IC who calls MC who sends the engineer. How it actually works is that the MC will not honor the purchase orders of the IC, so we are forced to do it another way. We have to inform both IC and MC of our need, we then generate a PO# for MC, MC services the scope, we pay MC and IC reimburses us. I personally only have to do the first part, but other labs may have to do the PO generation and reimbursement paperwork. So here are the pros and cons . . . Pros—1) We get a reduced price for a service contract. 2) The service comes from the same MC as before, so we are not reducing service quality. 3) We get to appease the Deans. Cons—1) More paper work for us. 2) Takes some time to generate PO# (depending upon your accounting system). 3) You are not top priority on the MC list (i.e., slower response time). So, it really comes down to whether you think the increase in paperwork and slower response time is worth the money you save. Some have the resources, others do not (especially in these times). Interestingly, the MCs stand to gain by having people pay per diem and top dollar for parts. The microscopy community may benefit from competition on pricing. I am not trying to convince anyone to go either way, but I thought it was important to properly describe this whole process and let managers decide upon facts. Ken Livi klivi@jhu.edu Wed Oct 27

I wholeheartedly agree with Gary and the other posters who warned of the downside of insurance contracts versus OEM service. About 5 years ago one of the reputable insurance companies in the field convinced my management that we could save at least 15% by letting them underwrite the policies. The folks at the insurance company were helpful and processed the paperwork promptly. We discovered that the service on our FEG SEM and TEMs were the largest expenditure for our analytical department. One major problem was that the vendor could not ship boards until a PO for the cost was received from the insurance company. This required a quote for the part and made it hard to get boards shipped out the same afternoon. Frequently the engineer has it narrowed down to a couple of boards. Under contract an engineer would get whatever parts might be needed for the next day sent out overnight and send back the unused ones. This was much more difficult under the insurance contract, because quotes would be needed for each part and the insurance company was understandably cost conscious. As others have noted, contract customers take priority. This was an issue for us. The final straw was that it looked like we needed an HV tank for our 200 kV TEM. There was an exclusion for transformers in the insurance contract and my managers were not happy that we would be on the hook. This time it turned out to be a cable that was covered, but it could have been a major unplanned expense. We finally put together a business justification to put our TEM and FEG-SEM back under vendor contract. Actually everybody was pleased with this. The insurance company is a good choice for instrumentation that does not need service frequently and does not use very expensive parts. We could easily choose alternate vendors when it made sense. We used this option with our microtomes. Another benefit of working with the insurance company was that we had put in an allotment for consumables. When we needed LaB6 cathodes and apertures, the microscope vendor could ship and the insurance vendor would charge it to the right account—without us having to generate a purchase order. As I said, I am pleased with the insurance company for auxiliary equipment but think vendor service is best for high end SEMs and TEMs. The last point I will make, when considering an insurance provider, is whether your microscope vendor will accept their POs or if you have to write a company PO and get reimbursed. At least one major microscope manufacturer will not accept POs from many insurance companies. They had too many problems collecting. The company we used had a history of prompt payment, so the microscope company agreed to continue to take their POs. People considering such options should have a frank discussion with the service manager for their microscope vendor. John Minter jrminter@rochester.rr.com Wed Oct 27

MT

https://doi.org/10.1017/S1551929510001148 Published online by Cambridge University Press